MycoKeys 110: 141–158 (2024)
DOI: 10.3897/mycokeys.110.135724

Research Article

Two new *Cordyceps*-like species, *Perennicordyceps zongqii* sp. nov. (Polycephalomycetaceae) and *Purpureocillium zongqii* sp. nov. (Ophiocordycipitaceae), in Hypocreales from karst region of China

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Abstract

Two new *Cordyceps*-like species, *Perennicordyceps zongqii* and *Purpureocillium zongqii*, isolated from a larva and soil, are introduced. Morphological comparisons and phylogenetic analyses based on multigene datasets (ITS, LSU, *RPB2* and *TEF*) support the establishment of the new species. Moreover, new species in the families Polycephalomycetaceae and Ophiocordycipitaceae were introduced into Tiankeng and the valley for the first time. Further attention needs to be paid to the diversity of other *Cordyceps*-like fungi in the special eco-environment of the karst region.

Key words: *Cordyceps*-like species, morphology, Ophiocordycipitaceae, phylogenetic analysis, Polycephalomycetaceae

OPEN ACCESS

Academic editor: Marc Stadler
Received: 28 August 2024
Accepted: 21 October 2024
Published: 7 November 2024

Citation: Chen W-H, Li D, Liang J-D, Ren X-X, Zhao J-H, Han Y-F (2024) Two new *Cordyceps*-like species, *Perennicordyceps zongqii* sp. nov. (Polycephalomycetaceae) and *Purpureocillium zongqii* sp. nov. (Ophiocordycipitaceae), in Hypocreales from karst region of China. MycoKeys 110: 141–158. https://doi.org/10.3897/mycokeys.110.135724

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Introduction

Cordyceps-like fungi, also known as *Cordyceps* sensu lato, refers to species belonging to Hypocreales, Sordariomycetes and Ascomycota, and contains all the species in the families Cordycipitaceae, Ophiocordycipitaceae and Polycephalomycetaceae, as well as some species in the family Clavicipitaceae (Li et al. 2021; Xiao et al. 2023). Currently, more than 2000 species of *Cordyceps*-like fungi have been reported worldwide, while there are just over 300 species in China (Chen et al. 2021d; Li et al. 2023, http://www.indexfungorum.org/Names/Names.asp, 17 August, 2024). Therefore, more attention needs to be paid to the diversity of *Cordyceps*-like fungi in China.

The karst region, especially in southern China, preserves unique, large-scale, and continuously distributed primitive forests with extremely rich biodiversity. The complex ecological environment and special geographical conditions in the region have become shelters for many unique species (Özkan et al. 2010; Su et al. 2017). Previous studies have shown that the resources of *Cordyceps*-like fungi in karst areas are very abundant (Zhu et al. 2004; Song et al. 2011). In recent years, Yunnan and Guizhou Province have become hot areas for research on *Cordyceps*-like fungi

(Ming et al. 2021; Chen et al. 2021a, b, c, 2022a, b, c, 2023; Qu et al. 2021; Dong et al. 2022; Wang et al. 2022, 2024a; Zhou et al. 2022; Zou et al. 2022; Peng et al. 2023, 2024; Tang et al. 2023a, b, c; Xiao et al. 2023, 2024; Zhang et al. 2023; Dai et al. 2024), and the majority of sampling locations were located in karst forest habitats. However, there are also some special habitats in karst areas, such as Tiankeng and the valley, because of its unique geological landscape, which creates a microclimate distinct from its surrounding area and a unique habitat suitable for biological survival. Unfortunately, the *Cordyceps*-like fungi in these habitats have been neglected.

Chen et al. (2022b, 2023) reported ten new *Cordyceps*-like fungi in the family Cordycipitaceae from Tiankeng and the valley. Two new genera and three new species in the family Clavicipitaceae were introduced from the valley by Chen et al. (2022a). However, *Cordyceps*-like fungi were rarely reported in other families from Tiankeng or the valley. Besides, *Cordyceps*-like fungi was omnipresent in the obtaining nutrients and was abundant in the soil (Chen et al. 2022a, Quesada-Moraga et al. 2007). Unfortunately, few reports exist about the *Cordyceps*-like fungi from the soil of Tiankeng or the valley.

During a survey of *Cordyceps*-like fungi associated with insects and soil from Southwest China, the infected specimen and soil were collected, and strains were isolated. After detailed multiloci phylogenic analysis and morphological observations, two new species were identified as belonging to the family Polycephalomycetaceae and Ophiocordycipitaceae.

Materials and methods

Specimen and soil collection, isolation

The specimen and soil (for the photo descriptions of the sampling site see Suppl. materials 1–5) were collected from Mayao River Valley (26°22'8.3748"N, 107°23'16.96"E), Duyun City, Qiannan Buyei and Miao Autonomous Prefecture and Monkey-Ear Tiankeng (27°5'12.138"N, 107°0'48.42"E), Kaiyang County, Guiyang, Guizhou Province, on 1 May 2022 and 19 July 2023. The samples were placed in an ice box and brought to the laboratory. Specimens were preserved in the refrigerator at 4 °C until further processing. The surface of each arthropod body was rinsed with sterile water, followed by sterilization with 75% ethanol for 3–5 s and rinsing again three times with sterilized water. After drying on sterilized filter paper, a piece of the synnemata, mycelium or sclerotia was cut from the specimen and inoculated on agar plates of potato dextrose agar (PDA) or PDA modified by the addition of 1% w/v peptone containing 0.1 g/l streptomycin and 0.05 g/l tetracycline (Chen et al. 2019). After fungal colonies emerged from the inoculated samples, a piece of mycelium from the colony edge was transferred onto new agar plated and cultured at 25 °C for 14 days under 12 h light/12 h dark conditions (Zou et al. 2010). Then 2 g collected soil were placed into a sterile conical flask containing 20 ml sterile water and thoroughly shaken using a Vortex vibration meter. Next, the suspension was diluted to a concentration of 10^{-3}. Subsequently, 1 ml of the diluted sample was added to a sterile Petri dish and mixed with Sabouraud's dextrose agar (SDA; peptone 10 g/l, dextrose 40 g/l, agar 20 g/l, 3.3 ml of 1% Bengal red aqueous solution) medium containing 50 mg/l penicillin and 50 mg/l streptomycin. After the plates were incubated at 25 °C for 1–2 weeks, single colonies were transferred

from the plates to new PDA plates (Wang et al. 2024b). The holotypes and ex-types were deposited at the Institute of Fungus Resources, Guizhou University (formally Herbarium of Guizhou Agricultural College; code, GZAC), Guiyang City, Guizhou, China. MycoBank numbers have been obtained as outlined in Myco-Bank (http://www.MycoBank.org) (Crous et al. 2004).

Morphological studies

Colony morphology was determined on PDA cultures incubated at 25 °C for 14 days and the growth rate, the presence of octahedral crystals and the colony colours (surface and reverse) were observed. To investigate the microscop-ic characteristics, a little of the mycelia was picked up from the colony and mounted in lactophenol cotton blue or 20% lactate acid solution and the asex-ual morphological characteristics (e.g., conidiophores, phialides and conidia) were observed and measured using a Leica DM4 B microscope. Twenty mea-surements were recorded for hyphae, conidiophores, phialides and conidium.

DNA extraction, PCR and sequencing

DNA extraction was carried out using a fungal genomic DNA extraction kit (DP2033, BioTeke Corporation) according to Liang et al. (2011). The extracted DNA was stored at −20 °C. Polymerase chain reaction (PCR) was used to amplify genetic markers using the following primer pairs: ITS4/ITS5 for the internal tran-scribed spacer (ITS) region (White et al. 1990), LR0R/LR5 for 28s large subunit ribosomal (LSU) (Vilgalys and Hester 1990), fRPB2-5F/fRPB2-7cR for RNA poly-merase II second largest subunit (*RPB2*) (Liu et al. 1999) and 983F/2218R for translation elongation factor 1 alpha (*TEF*) (Castlebury et al. 2004). The thermal cycle of PCR amplification for these phylogenetic markers was set up following the procedure described by Chen et al. (2021c). PCR products were purified and sequenced at Sangon Biotech (Shanghai) Co. by Sanger dideoxy sequencing. All newly generated sequences were deposited in GenBank and accession num-bers were obtained. The sequences used in the study were listed in Table 1.

Sequence alignments and phylogenetic analyses

DNASTAR™ Lasergene (v 6.0) was used to edit DNA sequences in this study. The ITS, LSU, *RPB2* and *TEF* sequences were downloaded from GenBank, based on Xiao et al. (2023), Calvillo-Medina et al. (2021), Luangsa-ard et al. (2011), Chen et al. (2024) and others selected based on BLASTn searches in GenBank. ITS sequences and other loci were aligned and edited by MAFFT v.7.037b (Katoh and Standley 2013) and MEGA6 (Tamura et al. 2013). Combined sequences of ITS, LSU, *RPB2* and *TEF* were obtained using SequenceMatrix v.1.7.8 (Vaidya et al. 2011). The model was selected for Bayesian analysis by ModelFinder (Kalyaana-moorthy et al. 2017) in PhyloSuite (v1.2.2) software (Zhang et al. 2020).

The combined dataset of ITS, LSU, *RPB2* and *TEF* sequence data (Suppl. materials 1–5) was analyzed using Bayesian inference (BI) and maximum likelihood (ML) methods. For BI, a Markov chain Monte Carlo (MCMC) algo-rithm was used to generate phylogenetic trees with Bayesian probabilities for the combined sequence datasets using MrBayes v.3.2 (Ronquist et al. 2012).

Table 1. List of strains and GenBank accession numbers of sequences used in this study.

Species	Strain	Host/ substrate	GenBank Accession Number				Reference
			ITS	LSU	RPB2	TEF	
Perennicordyceps cuboidea	NBRC 103836	Larva of beetle	JN943332	JN941420	AB972955	AB972951	Schoch et al. 2012
Perennicordyceps cuboidea	NBRC 100941	stroma of *Cordyceps stylophora*	JN943329	JN941416	–	–	Schoch et al. 2012
Perennicordyceps cuboidea	NBRC 101740	Larva of beetle	JN943331	JN941417	–	KF049684	Schoch et al. 2012
Perennicordyceps elaphomyceticola	MFLU 21-0264	*Elaphomyces* sp.	OQ172067	OQ172035	OQ459794	OQ459720	Xiao et al. 2023
Perennicordyceps elaphomyceticola	MFLU 21-0263	*Elaphomyces* sp.	OQ172065	OQ172033	OQ459793	OQ459719	Xiao et al. 2023
Perennicordyceps elaphomyceticola	MFLU 21-0262	*Elaphomyces* sp.	OQ172064	OQ172032	OQ459792	OQ459718	Xiao et al. 2023
Perennicordyceps lutea	KUMCC 3004^{T}	*Ophiocordyceps sinensis*	–	OQ474910	–	–	Xiao et al. 2023
Perennicordyceps paracuboidea	NBRC 101742^{T}	Larva of beetle	JN943338	JN941431	KF049669	KF049685	Ban et al. 2015a
Perennicordyceps paracuboidea	NBRC 100942	Larva of beetle	JN943337	JN941430	AB972958	AB972954	Ban et al. 2015a
Perennicordyceps prolifica	NBRC 101750	Larva of *Tanna japonensis*	JN943340	JN941433	AB972957	AB972953	Ban et al. 2009
Perennicordyceps prolifica	NBRC 100744	Larva of *Tanna japonensis*	AB925942	JN941432	AB972956	AB972952	Ban et al. 2009
Perennicordyceps prolifica	TNS-F-18547	Larvae of cicada	KF049660	KF049632	KF049670	KF049687	Kepler et al. 2013
Perennicordyceps ryogamiensis	NBRC 101751	Larva of beetle	JN943343	JN941438	–	KF049688	Schoch et al. 2012
Perennicordyceps ryogamiensis	NBRC 103837	Larva of beetle	JN943346	JN941439	–	–	Schoch et al. 2012
Perennicordyceps ryogamiensis	NBRC 103842	*Cordyceps ryogamiensis*	JN943345	JN941440	–	–	Schoch et al. 2012
Perennicordyceps zongqii	**DY05421^{T}**	**Larva of moth**	**PQ211278**	**PQ211282**	**PQ223677**	**PQ223679**	This study
Perennicordyceps zongqii	**DY05422**	**Larva of moth**	**PQ211279**	**PQ211283**	**PQ223678**	**PQ223680**	This study
Pleurocordyceps parvicapitata	MFLU 21-0270	*Elaphomyces* sp.	OQ172082	OQ172054	OQ459796	OQ459722	Xiao et al. 2019
Pleurocordyceps parvicapitata	MFLU 21-0271^{T}	*Elaphomyces* sp.	OQ172083	OQ172055	OQ459797	OQ459723	Xiao et al. 2023
Pleurocordyceps sinensis	HMAS 43720^{T}	Larvae of *Hepialus armocanus*	NR_119928	NG_042573	–	–	Sun et al. 2019
Pleurocordyceps vitellina	KUMCC 3006^{T}	*Ophiocordyceps nigrella*	OQ172089	OQ172061	OQ459803	OQ459729	Xiao et al. 2023
Pleurocordyceps vitellina	KUMCC 3007	*Ophiocordyceps nigrella*	OQ172090	OQ172062	OQ459804	OQ459730	Xiao et al. 2023
Polycephalomyces formosus	NBRC 109993^{T}	Larvae of Coleoptera	MN586833	MN586842	MN598064	MN598057	Wang et al. 2021
Polycephalomyces albiramus	GACP 21-XS08^{T}	Gryllotalpa	OQ172092	OQ172037	OQ459807	OQ459735	Xiao et al. 2023
Polycephalomyces albiramus	GACPCC 21-XS08	Gryllotalpa	OQ172093	OQ172038	OQ459808	OQ459736	Xiao et al. 2023
Purpureocillium atypicola	CBS 744.73	*Atypus karschi*	GU980041	EF468841	–	EF468786	Perdomo et al. 2013
Purpureocillium jiangxiense	JX17D04	Soil	PP555636	PP555645	–	PP658209	Chen et al. 2024
Purpureocillium jiangxiense	JX13B01^{T}	Soil	PP555637	PP555646	–	PP658210	Chen et al. 2024
Purpureocillium lavendulum	FMR 10376^{T}	Soil	FR734106	–	–	FR775516	Perdomo et al. 2013
Purpureocillium lavendulum	CBS 128678	Human	MH864977	MH876430	–	–	Perdomo et al. 2013
Purpureocillium lilacinum	CBS 284.36^{T}	Soil	FR734101	–	–	FR734156	Perdomo et al. 2013
Purpureocillium lilacinum	FMR 8652	Human	FR734090	FR775473	–	–	Perdomo et al. 2013
Purpureocillium roseum	IOM 325363.1	Human	MT560195	MT560197	–	–	Calvillo-Medina et al. 2021
Purpureocillium roseum	IOM 325363.2	Human	MT560196	MT560198	–	–	Calvillo-Medina et al. 2021
Purpureocillium sodanum	IBRC-M 30175^{T}	Salt crystals	KX668542	–	–	–	Hyde et al. 2016
Purpureocillium takamizusanense	NBRC 100742	*Tanna japonensis*	LC008197	–	–	LC008333	Ban et al. 2015b
Purpureocillium takamizusanense	NBRC 108982	Cicada	LC008204	–	–	LC008338	Ban et al. 2015b
Purpureocillium takamizusanense	NBRC 110232	-	LC008205	–	–	LC008339	Ban et al. 2015b
Purpureocillium zongqii	**TK041^{T}**	**Soil**	**PQ211280**	**PQ211284**	–	**PQ223681**	This study
Purpureocillium zongqii	**TK042**	**Soil**	**PQ211281**	**PQ211285**	–	**PQ223682**	This study
Simplicillium lamellicola	CBS 116.25^{T}	*Agaricus bisporus*	MH854806	AF339552	DQ522462	DQ522356	Luangsa-ard et al. 2011
Simplicillium lanosoniveum	CBS 704.86	*Hemileia vastatrix*	AJ292396	AF339553	DQ522464	DQ522358	Luangsa-ard et al. 2011

Note: New strains or species are in bold type."T" denotes ex-type. Abbreviations for collections: CBS, Centraalbureau voor Schimmelcultures, Utrecht, the Netherlands; TNS-F, the mycological herbarium of the National Museum of Nature and Science, Tsukuba, Ibaraki, Japan; HMAS, Herbarium of Mycology, Chinese Academy of Sciences; IBRC, Iranian Biological Resource Center, Tehran, Iran; NBRC, NITE Biological Resource Center, Sayaka Ban National Institute of Technology and Evaluation, Japan; IOM, Instituto de Oftalmología 'Fundación Conde de Valenciana' IAP Mexico Culture Collection; MFLU, Mae Fah Luang University; GACP, Herbarium of Guizhou University, China; KUMCC, Culture collection of Kunming Institute of Botany, Kunming, China; FMR, Facultad de Medicina, Reus, Tarragona, Spain.

The Bayesian analysis resulted in 20,001 trees after 10,000,000 generations. The first 4,000 trees, representing the burn-in phase of the analysis, were discarded, while the remaining 16,001 trees were used to calculate posterior probabilities in the majority rule consensus tree. After the analysis was finished, each run was examined if it was greater than 200 using the program Tracer v.1.5 (Drummond and Rambaut 2007) to determine burn-in and confirm that both runs had converged. ML analyses were constructed with IQ-TREE (v 2.0) (Trifinopoulos et al. 2016), using an automatic selection of the model according to BIC.

Results

Phylogenetic analyses

The phylogenetic tree (Fig. 1) was generated to determine the relationship among those new strains and its related species. *Simplicillium lanosoniveum* (J.F.H. Beyma) Zare & W. Gams (CBS 704.86) and *S. lamellicola* (F.E.V. Sm.) Zare & W. Gams (CBS 116.25) were used as the outgroups. The concatenated sequences included 40 taxa and consisted of 3,392 (ITS, 616; LSU, 841; *RPB2*, 1,041; and *TEF*, 894) characters with gaps.

The selected model for ML analysis was TN+F+I+G4. The final value of the highest scoring tree was −15,988.941, which was obtained from an ML analysis of the dataset (ITS, LSU, *RPB2* and *TEF*). The parameters of the rate heterogeneity model used to analyze the dataset were estimated using the following frequencies: A = 0.230, C = 0.288, G = 0.278, T = 0.204; substitution rates AC = 1.00000, AG = 2.57904, AT = 1.00000, CG = 1.00000, CT = 5.70221 and GT = 1.00000, as well as the gamma distribution shape parameter α = 0.805. The selected models for BI analysis were GTR+F+I+G4 (ITS, LSU, *RPB2* and *TEF*). The phylogenetic trees (Fig. 1) constructed using ML and BI analyses were largely congruent and strongly supported in most branches. The new strains DY05421 and DY05422 were clustered into an independent clade in the group of the genus *Perennicordyceps* and have a close relationship with *Perennicordyceps cuboidea* (Kobayasi & Shimizu) Matočec & I. Kušan (NBRC 101740, NBRC 103836 and NBRC 100941) and *P. ryogamiensis* (Kobayasi & Shimizu) Matočec & I. Kušan (NBRC 101751, NBRC 103842 and NBRC 103837). Strains TK041, TK042 were clustered into the group of the genus *Purpureocillium* and have a close relationship with *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (CBS 284.36).

Taxonomy

***Perennicordyceps zongqii* W.H. Chen, Y.F. Han & J.D. Liang, sp. nov.**

MycoBank No: 855564

Fig. 2

Etymology. In honor of Prof. Zongqi Liang, for his support and guidance in arthropod pathogenic fungi research.

Type. China • Guizhou Province, Qiannan Buyei and Miao Autonomous Prefecture, Duyun City, Mayao River Valley (26°22'8.3748"N, 107°23'16.96"E), on a larva of moth (Lepidoptera), on the leaf litter, 1 May 2022, Wanhao Chen, GZAC DY0542 (holotype), ex-type DY05421.

Figure 1. Phylogenetic analysis of the new strains and its related species based on multigene dataset (ITS, LSU, *RPB2* and *TEF*). Statistical support values (≥ 50%/0.5) are shown at the nodes for ML bootstrap support/BI posterior probabilities.

Description. Colonies on PDA, attaining a diameter of 56–59 mm after 14 days at 25 °C, white, consisting of a basal felt, floccose hyphal overgrowth, yellowish in middle; reverse yellow to pale yellowish, light brown to brown in the middle. Hyphae septate, hyaline, yellowish in the middle part of the colony, smooth-walled, 1.1–2.1 µm wide. Conidiophores erect, hyaline, irregular

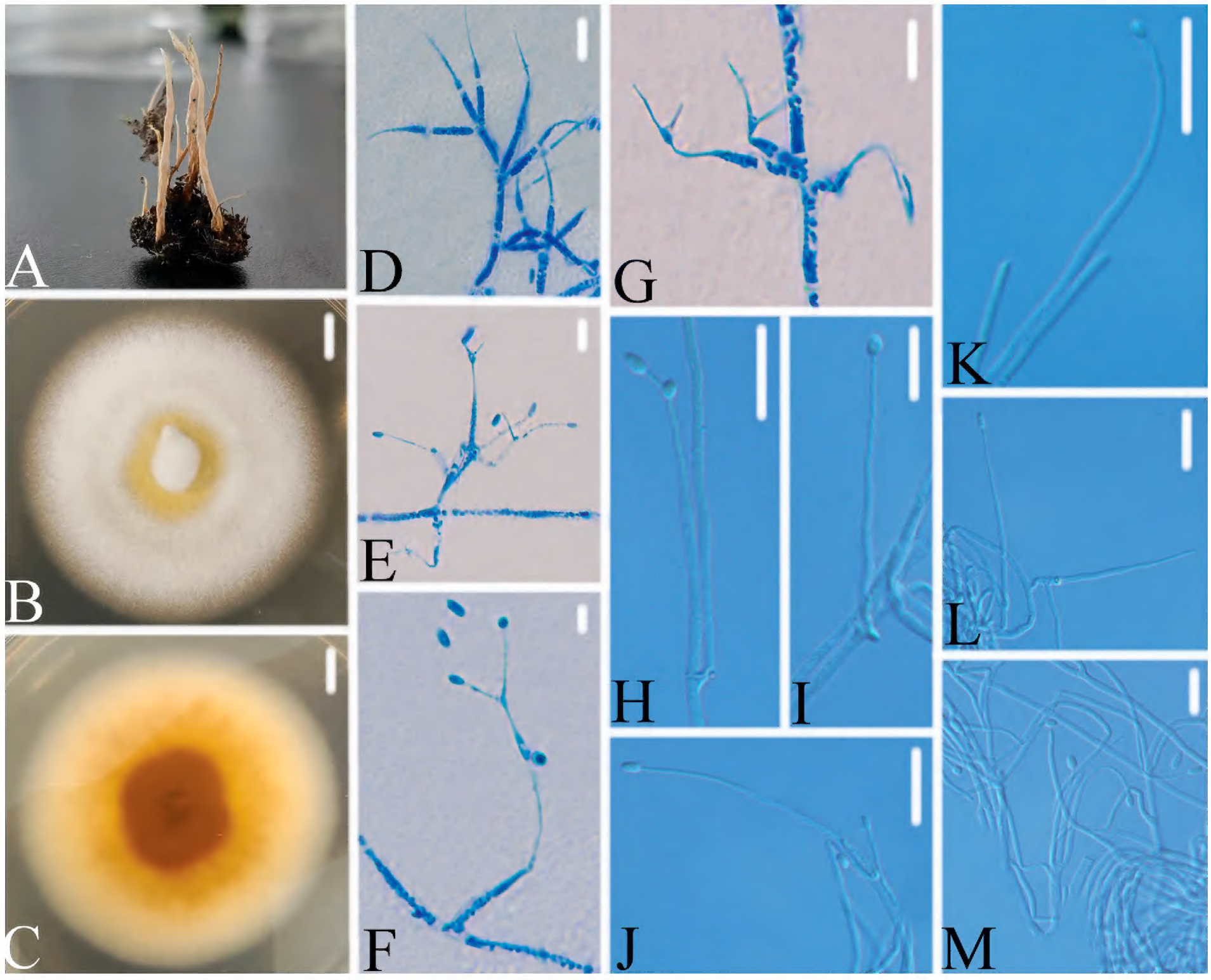

Figure 2. *Perennicordyceps zongqii* **A** infected larva **B**, **C** colony on PDA (**B** obverse, **C** reverse) **D–M** phialides and conidia. Scale bars: 10 mm (**B**, **C**); 10 μm (**D–M**).

branched, with 1–4 phialides. Phialides 29.3–31.1 × 1.5–2.4 μm, hyaline, cylindrical at base, gradually tapering near the apex, holoblastic or branch. Conidia 3.4–4.8 × 2.5–2.7 μm, hyaline, smooth-walled, thin-walled, ellipsoidal to cylindrical, unicellular, acuminate, arranged in chains not observed.

Distribution. Duyun City, Guizhou Province, China.

Host. Larva (Lepidoptera).

Additional strain examined. CHINA • Guizhou Province, Qiannan Buyei and Miao Autonomous Prefecture, Duyun City, Mayao River Valley (26°22'8.3748"N, 107°23'16.96"E). On a larva of moth (Lepidoptera), on the leaf litter, 1 May 2022, Wanhao Chen, DY05422 (living culture).

Notes. Strain DY05421 was easily identified as *Perennicordyceps*, based on the BLASTn result in NCBI. Phylogenetic analyses show that strain DY05421 has close relationships to *P. cuboidea and P. ryogamiensis* (Fig. 1). However, strain DY05421 was easily distinguished from *P. cuboidea* (globose to ellipsoid conidia, 1.3–3.7 × 1.1–2.3 μm; phialide, 18.9–22.2 × 0.8–1.1 μm; substrate, larvae of Coleoptera) by its larger ellipsoidal to cylindrical conidia, larger phialide and the substrate (Ban et al. 2009). Strain DY05421 was easily distinguished from *P. ryogamiensis* (ellipsoid conidia, 2.5–3.9 × 1.0–1.4 μm; phialide, 17.5–55.2 ×

0.8–2.7 µm; substrate, larvae of Coleoptera) by its larger ellipsoidal to cylindrical conidia, smaller phialide and the substrate (Ban et al. 2009). Thus, the morphological characteristics and molecular phylogenetic results support *strain* DY05421 as a new *Perennicordyceps* species and named *Perennicordyceps zongqii*.

Purpureocillium zongqii **W.H. Chen, Y.F. Han & J.D. Liang, sp. nov.**

MycoBank No: 855565

Fig. 3

Etymology. In honor of Prof. Zongqi Liang, for his support and guidance in arthropod pathogenic fungi research.

Type. CHINA • Guizhou Province, Guiyang, Kaiyang County, Monkey-Ear Tiankeng (27°5'12.138"N, 107°0'48.42"E), soil, 19 July 2023, Wanhao Chen, GZAC TK04 (dried holotype), ex-type TK041.

Description. Colonies on PDA, attaining a diameter of 23–25 mm after 14 days at 25 °C, white, consisting of a basal felt, floccose hyphal overgrowth, white; reverse yellowish. Hyphae septate, hyaline, smooth-walled, 1.1–1.9 µm wide. Conidiophores 9.3–12.7 × 2.1–2.4 µm, erect, hyaline, verticillately branched, with 1–4 phialides. Phialides 6.8–11.7 × 2.6–4.0 µm, hyaline, cylindrical at base, gradually tapering near the apex. Conidia 2.7–4.2 × 2.0–2.4 µm, hyaline, smooth-walled, thin-walled, ellipsoidal, unicellular, acuminate, arranged in chains not observed.

Substrate. Soil.

Distribution. Kaiyang County, Guizhou Province, China.

Additional strain examined. CHINA • Guizhou Province, Guiyang, Kaiyang County, Monkey-Ear Tiankeng (27°5'12.138"N, 107°0'48.42"E), soil, 19 July 2023, Wanhao Chen, TK042 (living culture).

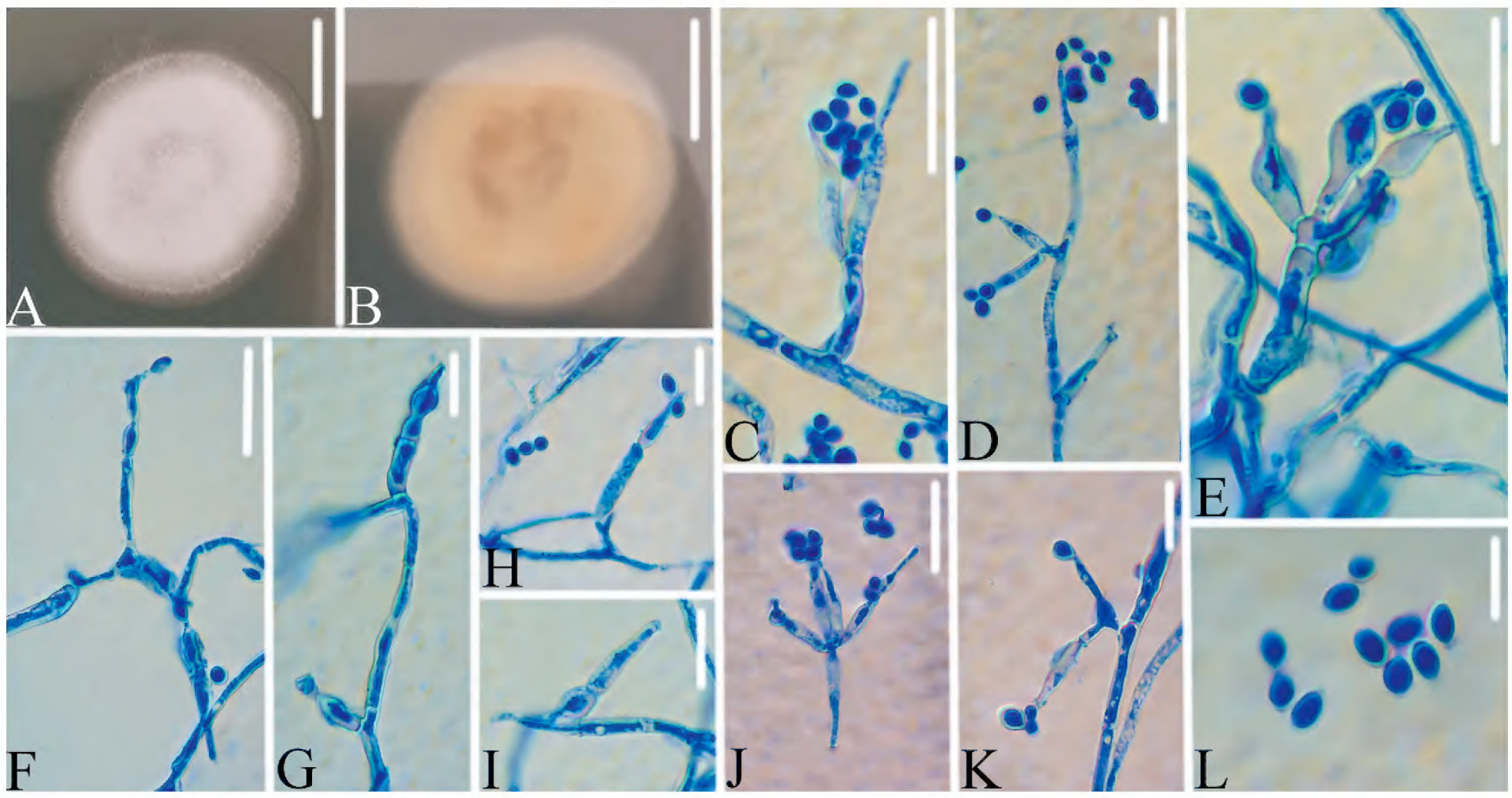

Figure 3. *Purpureocillium zongqii* **A**, **B** colony on PDA (**A** obverse, **B** reverse) **C–J** Phialides and conidia. Scale bars: 10 mm (**A**, **B**); 10 µm (**C–J**).

Notes. *Purpureocillium zongqii* was easily identified as *Purpureocillium*, based on the BLASTn result in NCBI. Phylogenetic analyses show that *P. zongqii* has a close relationship to *P. lilacinum* (Fig. 1). However, *P. zongqii* was easily distinguished from *P. lilacinum* (conidiophores: 4–6 × 3–4 µm; conidia: ellipsoidal to fusiform, 2–3 × 2–4 µm; phialide, 6–9 × 2.5–3 µm; purple colony) (Luangsa-ard et al. 2011) by its larger conidiophores, larger ellipsoidal conidia, larger phialide and white colony. Thus, the morphological characteristics and molecular phylogenetic results support *P. zongqii* as a new species.

Keys of the genus *Perennicordyceps*

1 Parasitic on fungi **2**
– Parasitic on insects **3**
2 Phialides 14.8–64.9 × 1.9–3.1 µm, conidia globose to ellipsoid ..... ***Perennicordyceps lutea***
– Phialides 12–16 × 0.6–1.5 µm, conidia fusiform to ellipsoid to inequilateral shaped ..... ***Perennicordyceps elaphomyceticola***
3 Typical host larva of cicada or moth **4**
– Typical host larva of beetle **5**
4 Typical host larva of cicada, conidia globose, fusiform 1.5–3.5 (2.5) × 1.1–1.8 (1.4) µm ..... ***Perennicordyceps prolifica***
– Typical host larva of moth, conidia ellipsoidal to cylindrical, 3.4–4.8 × 2.5–2.7 µm ..... ***Perennicordyceps zongqii***
5 Conidiophores quasi-verticillate branching **6**
– Conidiophores irregular branching ..... ***Perennicordyceps cuboidea***
6 Conidia ellipsoid, 2.5–3.9 (3.1) × 1.0–1.4 (1.2) µm, mainly *Acremonium*-like phialide ..... ***Perennicordyceps ryogamiensis***
– Conidia aubglobose, fusiform, 1.3–1.9 (1.8) × 1.0–1.9 (1.4) µm, mainly *Hirsutella*-like phialide ..... ***Perennicordyceps paracuboidea***

Keys of the genus *Purpureocillium*

1 Species that grow on spiders and insect forming synnemata of colours purple to lilac **2**
– Species isolated, clinical specimens on human, animals, soil and crystals salt **3**
2 Synnemata lilac coloured, conidia ellipsoid to cylindrical 4.8–5.6 × 1.6–2.4 µm; parasite trapdoor spiders; sexual state *Cordyceps cylindrica* ..... ***Purpureocillium atypicola***
– Synnemata lilac coloured, conidia ellipsoid 2.5–4 × 1.4–1.8 µm; parasite on cadavers of cicada adults; sexual state in *Cordyceps ryogamimontana* ..... ***Purpureocillium takamizusanense***
3 *Acremonium*-like synanamorph absent **4**
– *Acremonium*-like synanamorph present **5**
4 Phialides 8–10 (14) × 2–3 µm, conidia globose, 2–2.5 µm ..... ***Purpureocillium roseum***
– Phialides 6.8–11.7 × 2.6–4.0 µm, conidia ellipsoidal, 2.7–4.2 × 2.0–2.4 µm ..... ***Purpureocillium zongqii***

5 Conidia subglobose with apiculate base or limoniform **6**
– Conidia ellipsoidal to fusiform ..... ***Purpureocillium lilacinum***
6 Conidia 2–3 × 2–2.5 µm ..... ***Purpureocillium lavendulum***
– Conidia 3.5–5.5 × 3–4.5 µm ..... ***Purpureocillium sodanum***

Discussion

The genus *Perennicordyceps* was proposed to accommodate four species of *Polycephalomyces*, *Polycephalomyces prolificus* (Kobayasi) Kepler & Spatafora, *P. cuboideus* (Kobayasi & Shimizu) Kepler & Spatafora, *P. paracuboideus* (S. Ban, Sakane & Nakagiri) Kepler & Spatafora and *P. ryogamiensis* (Kobayasi & Shimizu) Kepler & Spatafora (Matočec et al. 2014). Species in the genus usually known as entomopathogenic fungi with host in the orders Coleoptera and Hemiptera (Matočec et al. 2014). Xiao et al. (2023) reported a new species, *Perennicordyceps lutea* Y.B. Wang, H. Yu & Y.P. Xiao and a new combined species, *P. elaphomyceticola* (W.Y. Chuang, H.A. Ariyaw., J.I. Yang & Stadler) Y.P. Xiao & K.D. Hyde, which were both recorded as fungicolous. In the present study, the new species *P. zongqii* with Lepidoptera larvae was introduced. *Cordyceps*-like fungi have evolved adaptively through nutrient exchange and the ultimate goal is in the search of ideal food (Moonjely et al. 2016; Vidhate et al. 2023). Whether the new species has coevolved with its hosts and has special metabolizing processes is worthy of further research.

The genus *Purpureocillium* was established to accommodate *Paecilomyces lilacinus* Thom (Luangsa-ard et al. 2011). Members of *Purpureocillium* have a global distribution, especially for the type species *P. lilacinum*, which is commonly isolated from soil, decaying vegetation, insects, nematodes and laboratory air (as contaminant) (Luangsa-ard et al. 2011; Perdomo et al. 2013; Quandt et al. 2014; Ban et al. 2015b; Calvillo-Medina et al. 2021). In the present study, the new species *P. zongqii* was isolated from soil in Monkey-Ear Tiankeng. Tiankeng acts as a refugium for biodiversity amid changing global climate and some ancient (*Alsophila spinulosa* (Wall. ex Hook.) R. M. Tryon) and unique plants (cool-adapted plants) were present in Tiankeng (Shui et al. 2015; Bátori et al. 2017; Pu et al. 2019; Shen et al. 2020). Whether the new species is more ancient than others, has coevolved with its environment and has special metabolizing processes is worthy of further research.

The karst region in southwestern China is one of the 36 biodiversity hotspots in the world, nurturing a large number of endemic species (Delgado Baquerizo et al. 2020), especially in the special eco-environment, Tiankeng and the valley. The species that exist in these habitats often have very narrow distribution areas and very small populations, and urgently need to be protected (Wu and Zhang 2020). In the present study, species in the families Polycephalomycetaceae and Ophiocordycipitaceae were introduced in Tiankeng and the valley for the first time. Further attention needs to be paid to the diversity of other *Cordyceps*-like fungi in the special eco-environment of karst region.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Funding

This work was funded by National Natural Science Foundation of China (31860002, 81960692), High-level Innovative Talents Training Object in Guizhou Province (Qiankehepingtairencai [2020]6005), Construction Program of Key Laboratory of Guizhou Province (Qiankehepingtairencai-ZDSYS[2023]004), Research Center Project of Guizhou University of Traditional Chinese Medicine (Guizhongyi ZX hezi [2024]021).

Author contributions

Data curation: WHC. Formal analysis: XXR, JDL. Funding acquisition: YFH, JHZ, WHC. Methodology: YFH. Resources: WHC, DL. Writing - original draft: JDL, WHC, XXR, DL. Writing - review and editing: YFH, JHZ.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

Alignment dataset 1

Authors: Wan-Hao Chen, Dan Li, Jian-Dong Liang, Xiu-Xiu Ren, Jie-Hong Zhao, Yan-Feng Han

Data type: fas

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Link: https://doi.org/10.3897/mycokeys.110.135724.suppl1

Supplementary material 2

Alignment dataset 2

Authors: Wan-Hao Chen, Dan Li, Jian-Dong Liang, Xiu-Xiu Ren, Jie-Hong Zhao, Yan-Feng Han

Data type: fas

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Supplementary material 3

Alignment dataset 3

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Data type: fas

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Link: https://doi.org/10.3897/mycokeys.110.135724.suppl3

Supplementary material 4

Alignment dataset 4

Authors: Wan-Hao Chen, Dan Li, Jian-Dong Liang, Xiu-Xiu Ren, Jie-Hong Zhao, Yan-Feng Han

Data type: fas

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Supplementary material 5

Alignment dataset 5

Authors: Wan-Hao Chen, Dan Li, Jian-Dong Liang, Xiu-Xiu Ren, Jie-Hong Zhao, Yan-Feng Han

Data type: nxs

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